Analysis of Native Microflora and Selection of Strains Antagonistic to Human Pathogens on Fresh Produce†

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ABSTRACT

The native microflora of three types of produce (green bell peppers, Romaine lettuce, and prepeeled baby carrots) and two types of sprouting seeds (alfalfa and clover) were investigated. Aerobic plate count (APC) for each produce or seed type as determined on *Pseudomonas* agar F (PAF) with incubation at 28°C was in the range of 4 to 7 log CFU per g of tissue or seed. There was no significant difference ($P \geq 0.05$) in APC when the determinations were made with three agar media including PAF, brain heart infusion agar, and plate count agar. However, the APC as determined from plates that were incubated at 28°C was significantly ($P \leq 0.05$) higher than with incubation at 37°C. Fluorescent pseudomonads accounted for 23 to 73% of APC and 6 to 18% of APC recovered from carrots, pepper, and lettuce were pectolytic. Forty-eight strains of pectolytic bacteria were randomly isolated and identified, respectively, as members of the genera of *Pseudomonas, Erwinia, Bacillus, Xanthomonas,* or *Flavobacterium.* Lactic acid bacteria and/or yeast were consistently isolated from baby carrots, lettuce, and sprouting seeds (alfalfa or clover) but not from green bell peppers. Approximately 120 strains of indigenous microflora were tested for their ability to inhibit the growth of *Salmonella* Chester, *Listeria monocytogenes, Escherichia coli,* or *Erwinia carotovora* subsp. *carotovora* on PAF. Six isolates capable of inhibiting the growth of at least one pathogen were isolated and identified, respectively, as *Bacillus* spp. (three strains), *Pseudomonas aeruginosa* (one strain), *Pseudomonas fluorescens* (strain A3), and yeast (strain D1). When green pepper disks were inoculated with strains A3 and D1, the growth of *Salmonella* Chester and *L. monocytogenes* on the disks was reduced by 1 and 2 logs, respectively, over a period of 3 days. Application of strains A3 and D1 as potential biopreservatives for enhancing the quality and safety of fresh produce is discussed.

It is generally believed that the indigenous microflora of fresh fruits and vegetables are very different from those found on food products of animal origins such as beef, pork, and poultry. Culture methods and conditions as described in the U.S. Food and Drug Administration *Bacteriological Analytical Manual (27)* are frequently used as a guide to enumerate the total aerobic plate counts (APC) of animal and dry food products. The appropriateness of using the same guide or procedure to enumerate the indigenous microflora on fresh produce has not yet been carefully evaluated. So far, there is no standard method or procedure available for enumeration of native APC on fresh produce. In previous studies, different types of culture media including plate count agar *(2, 4, 11, 17),* tryptic soy agar *(1, 3),* and Levure peptone glucose agar *(5, 6, 25)* as well as different incubation temperatures including 35 *(2),* 30 *(1, 3, 11),* 25 *(5, 17, 25),* and 20°C *(4, 12)* have been employed to determine the APC of fresh produce. However, no study has been conducted to determine if incubation at different temperatures and cultivation on different media may affect the determination of APC and the recovery of native microflora from fresh produce.

Native microflora naturally present on the surfaces of fresh produce are assumed to play an important role in maintaining the quality and safety of fresh and fresh-cut fruits and vegetables *(24).* Some of them are capable of producing antimicrobial compounds *(10, 15, 16, 22)* or activating plant defense mechanisms *(23),* leading to the suppression of growth of human pathogens on plant food products and, thus, are considered beneficial. Other native microflora are capable of inducing spoilage or off-flavors on fresh or packaged produce products and are considered deleterious *(23, 24).* A better understanding of the beneficial and deleterious microflora on fresh produce would provide a basis to develop more effective methods for extending the shelf life and enhancing the safety of fresh produce.

The objectives of this study were to (i) investigate the effect of incubation time and culture media on the recovery of native microflora from three types of produce (Romaine lettuce, prepeeled baby carrot, and green bell pepper) and two types of sprouting seeds (alfalfa and clover); (ii) determine the APC of each produce and identify special groups of native microflora, including pectolytic fluorescent pseudomonads, lactic acid bacteria (LAB), and yeasts that may affect the shelf life of fresh produce; and (iii) select native antagonists exhibiting growth inhibition against human pathogens and spoilage-causing bacteria on agar media and on fresh produce.

MATERIALS AND METHODS

Fresh produce and sprouting seeds. Alfalfa seed (lot no. 6NM-249) and clover seed (lot no. Y2-8-CC25) were obtained

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† Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

from Caudill Seed Co. (Louisville, Ky.) and International Specialty Supplies Inc. (Cookville, Tenn.), respectively. Packs of Romaine lettuce, prepeeled baby carrots, and green bell peppers were purchased from local retailers. They were transported to the laboratory and used for microbiological analyses on the same date when they were purchased. The ages of Romaine lettuce and prepeeled baby carrots used in the study were 3 to 5 and 7 to 10 days prior to the expiration date for consumption suggested by the manufacturer, respectively. Alfalfa and clover seeds were purchased in May 1999 and were stored at 4°C before use.

Enumeration and characterization of native microflora. Fresh produce or sprouting seeds (25 g) were homogenized in 125 ml of sterile phosphate-buffered saline (PBS, pH 7.2) using a Stomacher (model 400; Seward Medical Ltd., London, England) at high speed for 2 min for fresh produce samples or at low speed for 1 min for seed samples. Before homogenization, alfalfa and clover seeds were presoaked in PBS at 28°C for 2 h to facilitate the homogenization and recovery of native microflora from seeds. Tissue or seed homogenates were serially diluted in PBS and duplicate samples were plated on appropriate agar media and incubated at specified temperatures for determination of selected types of microflora. The APC was determined by plating the tissue or seed homogenate on *Pseudomonas* agar F (PAF; Difco, Detroit, Mich.), PCA (Difco), and brain heart infusion agar (BHIA; Difco) and incubated at 28°C for 3 days. To examine the effect of incubation temperatures on the recovery of native flora from produce samples, serially diluted homogenates were plated on PAF and incubated, respectively, at 37°C for 1 day, 28°C for 3 days, or 8°C for 7 days. Pectolytic microflora were identified by plating the tissue homogenates on semisolid pectate medium, and positive strains were indicated by the formation of a cavity surrounding the colony *(21)*. The population of LAB was enumerated by plating the tissue homogenate on deMan-Rogosa-Sharpe medium *(8)* and incubating the plates under anaerobic conditions at 20°C for 4 days. The number of yeasts in the sample was determined by plating the tissue homogenate on PCA supplemented with 200 μg/ml of chloramphenicol and incubating the plates at 28°C for 4 days.

Microbial identification. Pectolytic microbial isolates were randomly isolated from semisolid pectate medium and characterized according to their cellular morphology observed by phase-contrast microscopy (×1,000), their biochemical and physiological properties including Gram staining, oxidase and catalase tests, sporulation, budding, motility, and their ability to produce exopolysaccharide and pectinases. Biochemical and physiological tests were based on the methods previously described *(13, 26)*. Assays of pectate lyase and polygalacturonase were conducted according to the procedures previously described by Collmer et al. *(7)* and Liao et al. *(21)*. Microbial isolates antagonistic to human pathogens (see below) were characterized in the same way as described above. Final classifications of the potential biocontrol agents to the species level were performed at Midi Labs (Newark, Del.) based on 16S rRNA gene sequence similarity.

Isolation of native antagonists from fresh produce. Individual isolates of native microflora from each type of produce or seed were dotted on PAF using a sterile toothpick, six isolates per plate. After incubation at 28°C for 3 days, potential antagonists were killed by exposing the cultures to chloroform vapor for 30 min as described previously *(19)*. Culture plates were then overlaid with 3 ml of water agar (0.6%) containing 10^6 to 10^7 CFU/ml of the targeted bacterium, and the plates were incubated at 28°C for an additional 3 days. Antagonistic activity of each isolate was indicated by the formation of a growth inhibition zone (measured as mm in diameter) surrounding the colony. The pathogens or bacteria tested in the study included a rifampicin-resistant strain of *Listeria monocytogenes* (Scott A), a nalidixic acid–resistant strain of *Salmonella* Chester (ATCC 11997), and wild-type strains of *Escherichia coli* HB101 and *Erwinia carotovora* subsp. *carotovora* (SR319). With the exception of *E. carotovora* subsp. *carotovora* that was grown at 28°C, all the pathogens or bacteria were grown on BHIA at 37°C for routine cultivation and preparation of inoculum. If needed, rifampicin or nalidixic acid was added to the medium to a final concentration of 100 μg or 20 μg/ml, respectively.

Preparation of bacterial suspensions and assays of antagonists as potential biocontrol agents. For preparation of bacterial suspension for inoculation, *L. monocytogenes* or *Salmonella* Chester was grown on BHIA supplemented with rifampicin or nalidixic acid at 37°C for 1 day. The cells were harvested and suspended in PBS to the required cell density. Native microflora were prepared by inoculating lactose broth (Difco) with green pepper tissue homogenates and incubating the broth culture with shaking (125 rpm) at 28°C for 2 days. The culture was centrifuged (10,000 × *g*, 5 min), and the cell pellet was washed once with PBS and resuspended in PBS to obtain the required cell density.

Pepper disks were prepared from surface-sanitized fruits by using a sterile cork borer (no. 9, 154 mm^2 in cross section) as previously described *(20)*. Prepared disks were pooled together and inoculated with a bacterial suspension containing 10^6 CFU/ml of *L. monocytogenes* or *Salmonella* Chester and 10^8 CFU/ml of native microflora or individual antagonists. After air-drying on sterile cheesecloth for 10 min at room temperature, five disks were randomly selected from the pool, placed in a Stomacher bag, and used as a composite sample. In each experiment, three composite samples (or three bags) consisting of 15 disks were used. Disks inoculated with the pathogen alone (in the absence of microflora or antagonists) were used as control. Inoculated disks were incubated at 20°C for up to 4 days. Three composite samples were removed daily and the numbers of *L. monocytogenes* or *Salmonella* Chester on the disks were determined by plating the diluted tissue homogenates on BHIA medium containing rifampicin or nalidixic acid. Tissue homogenization was performed using a Seward Stomacher at high speed for 2 min. The reduction in the number of targeted pathogen in log CFU per disk was calculated by subtracting the number of targeted pathogen on the disks treated with an antagonist from the number of targeted pathogen on the disks treated with PBS.

Statistical analysis. All experiments were done in duplicate, and a minimum of three samples per experiment were analyzed. Analysis of variance and the Duncan multiple range test (SAS Institute, Inc., Cary, N.C.) were performed to determine significant difference on the logarithm of population densities. Significance was determined at the 0.05 level.

RESULTS AND DISCUSSION

Effects of culture media and incubation temperatures on the recovery of native microflora from fresh produce. The APC of each produce or sprouting seed as determined on three agar media showed no significant difference ($P \geq 0.05$) (Table 1). Although PCA was the medium most frequently used to enumerate the APC of food products *(2, 4, 11, 17)*, this study shows that either BHIA or PAF can serve as an alternative medium for enumeration of native microflora on fresh produce. In spite of its rich

TABLE 1. *Effect of culture media on the recovery of native microflora from fresh produce and sprouting seeds*[a]

	Total count (log CFU/g tissue or seed) on:		
	PAF	BHIA	PCA
Alfalfa seed[b]	4.21 ± 0.21 A[c]	4.36 ± 0.18 A	4.11 ± 0.27 A
Clover seed	4.56 ± 0.13 A	4.61 ± 0.08 A	4.32 ± 0.11 AB
Baby carrot	5.03 ± 0.13 A	4.98 ± 0.21 A	4.42 ± 0.17 A
Bell pepper	3.96 ± 0.23 A	3.84 ± 0.15 A	3.65 ± 0.26 A
Romaine lettuce	6.53 ± 0.31 A	6.21 ± 0.12 A	5.78 ± 0.22 AB

[a] Culture plates were incubated at 28°C for 3 days.
[b] Alfalfa and clover seeds were presoaked in PBS for 2 h at 28° C before homogenization.
[c] The value represents an average of three experiments ± standard deviation. In each experiment, two duplicates of each sample were used. Values within the same row that are followed by the same letter are not significantly different ($P \geq 0.05$).

composition, BHIA did not increase the recovery of native flora from fresh produce. Other rich media such as tryptic soy agar *(1, 3)* and Levure peptone glucose agar *(5, 6, 25)* have also been used to enumerate the resident microflora on other types of produce. All of these media appear to be adequate for enumerating APC on fresh produce, and it is not possible from any study to conclude if one medium is superior to the other. The PAF, a modification of the King's medium B *(18)*, was originally developed to propagate and enumerate fluorescent pseudomonads. This study indicates that PAF is suitable not only for enumeration of fluorescent pseudomonads but also for APC on fresh produce and sprouting seeds.

PAF plates containing serially diluted tissue homogenates were incubated, respectively, at 8, 28, and 37°C to determine the effect of incubation temperature on the determination of APC and recovery of native microflora from fresh produce. Table 2 shows that significantly higher numbers of native bacteria in all three produce and clover seed samples were found on culture plates that were incubated at 28°C than at 37°C. However, no significant difference in APC was seen for the alfalfa seed samples that were incubated at 28 and 37°C. In addition, a large number of native bacteria were detected on plates that were incubated at 8°C, indicating that substantial proportions of background bacteria are capable of growing on fresh produce stored at low temperatures. A vast majority of bacteria naturally associated with plants are unable to grow at temperatures higher than 37°C *(23)*. In previous studies of the APC from other produce, culture plates have been incubated at temperatures ranging from 20 to 30°C *(1, 4, 5)* and occasionally at 35°C *(2)*. This study suggests that culture plates for enumerating the APC of fresh produce should not be incubated at 37°C or higher.

Characterization of native microflora possibly affecting the shelf life of fresh produce. Table 3 shows that the APC of three produce and two sprouting seeds as determined on PAF with incubation at 28°C are in the range of 4 to 7 log CFU/g tissue or seed. Fluorescent pseudomonads accounted for 23 to 73% of APC on each produce. It has been previously reported that fluorescent pseudomonads constitute a major proportion of the indigenous microflora on several salad vegetables including spinach *(1)*, shredded carrots *(5)*, cabbage *(12)*, shredded iceberg lettuce *(17)*, and endive *(6)*. Here we found that fluorescent pseudomonads also represent a large proportion of native bacteria on sprouting seeds, prepeeled baby carrots, Romaine lettuce, and green bell peppers.

Pectolytic bacteria are generally believed to play an important role in causing the spoilage of fresh or freshcut salad vegetables *(3)*. Table 3 shows that pectolytic microflora accounted for 18, 6, and 13% of APC of prepeeled baby carrot, bell pepper, and Romaine lettuce, respectively. Forty-six strains of pectolytic bacteria were isolated and respectively identified as members of the genera of *Pseudomonas, Erwinia, Bacillus, Xanthomonas,* and *Flavobacterium* (Table 4). Among them, fluorescent pseudomonads

TABLE 2. *Effect of incubation temperatures on the recovery of native microflora from fresh produce and sprouting seeds*[a]

	Total count (log CFU/g tissue or seed)		
Produce	37°C	28°C	8°C
Alfalfa seed[b]	4.03 ± 0.12 A[c]	4.21 ± 0.20 A	3.85 ± 0.27 A
Clover seed	4.35 ± 0.10 A	4.61 ± 0.08 AB	4.10 ± 0.20 B
Baby carrot	4.28 ± 0.21 A	4.98 ± 0.14 B	4.27 ± 0.16 A
Bell pepper	3.88 ± 0.12 A	4.12 ± 0.07 B	3.95 ± 0.11 A
Romaine lettuce	5.43 ± 0.13 A	6.85 ± 0.21 B	5.13 ± 0.24 C

[a] Tissue or seed homogenates were plated on PAF (Difco).
[b] Alfalfa and clover seeds were presoaked in PBS at 28°C for 2 h before homogenization.
[c] The value represents an average of three experiments, two duplicates in each experiment ± standard deviation. Values within each row that are not followed by the same letter are significantly different ($P \leq 0.05$).

TABLE 3. *Enumeration of indigenous microflora from fresh produce and sprouting seeds*[a]

	Total count (log CFU/g)	% fluorescent pseudomonads	% pectolytic	Lactic acid bacteria (log CFU/g)	Yeast (log CFU/g)
Alfalfa seed	4.47 ± 0.21[b]	23	≤1	1.02 ± 0.20	ND
Clover seed	4.78 ± 0.13	73	≤1	ND	2.76 ± 0.17
Baby carrot	6.27 ± 0.31	36	18	3.21 ± 0.13	2.31 ± 0.21
Bell pepper	4.23 ± 0.27	26	6	ND	ND
Romaine lettuce	7.14 ± 0.13	38	13	1.24 ± 0.14	2.08 ± 0.18

[a] Total mesophilic count was determined by plating the tissue or seed homogenates on PAF (Difco) and incubating the plates at 28°C for 3 days. Alfalfa and clover seeds were presoaked in PBS at 28°C for 2 h prior to homogenization. Populations of fluorescent pseudomonads, pectolytic microflora, LAB, and yeasts were determined based on the methods described in the "Materials and Methods."

[b] The value represents an average of three experiments, two duplicates in each experiment ± standard deviation. ND, not detected.

were the most frequently isolated. More importantly, approximately 40% of the pectolytic fluorescent pseudomonads isolated were able to grow and produce fluorescent pigments at refrigeration temperature (≤4°C). It has been reported previously *(22)* that the ability of fluorescent pseudomonads to inhibit the growth of human pathogens such as *L. monocytogenes* on potato tuber slices is mainly due to its ability to produce fluorescent siderophores. Production of pectate lyase was detected in almost every strain of pectolytic bacteria analyzed. However, production of polygalacturonase was detected only in erwinias and certain strains of fluorescent pseudomonads. Possible involvement of pectolytic fluorescent pseudomonads in the development of spoilage of spinach *(1)*, endive *(6)*, cabbage *(12)*, lettuce *(2, 17)*, and green pepper *(3)* has been previously suggested. Besides pectolytic bacteria, LAB and yeasts have also been suspected as the cause of spoilage of shredded carrots *(5)* and grapes *(14)*. In this study, LAB and yeast were consistently isolated from prepeeled baby carrots and Romaine lettuce but not from green bell pepper. LAB and yeasts were also found to be present in alfalfa and clover seeds, respectively, but at relatively low numbers (Table 3). In addition, three pectolytic yeasts were isolated from baby carrots and shown to produce polygalacturonases similar to the ones previously reported *(9)*. The association of pectolytic yeasts and possibly LAB with the spoilage of fresh produce needs to be evaluated further.

TABLE 4. *Characterization of pectolytic bacteria from fresh produce and sprouting seeds*[a]

Characteristics	*Erwinia* (8)[b]	*Pseudomonas* (25)	*Bacillus* (4)	*Xanthomonas* (6)	*Flavobacterium* (5)
Gram stain	−	−	+	−	−
Fluorescence	−	+	−	−	−
Motility	+	+	+	+	−
Mucoidity	−	−	−	+	−
Sporulation	−	−	+	−	−
Gliding ability	−	−	−	−	+
Oxidase	−	+	−	−	+
Catalase	−	+	+	−	−
Growth at ≤4°C	±[c]	+	−	−	−
Pectate lyase	+	+	+	+	+
Polygalacturonase	+	±[d]	−	−	−

[a] Pectolytic colonies on the semisolid pectate medium were randomly selected for further characterization. Fluorescence and mucoidity were determined on PAF. +, positive; −, negative.

[b] The number of isolates analyzed. *Erwinia* spp. including *E. chrysanthemi* and *E. carotovora* subsp. *carotovora* were identified. The majority of *Pseudomonas* isolates were tentatively identified as *P. fluorescens*.

[c] ±, little or no growth.

[d] ±, four out of 25 strains analyzed produced polygalacturonase.

Selection of antagonists against human and plant pathogens from fresh produce. Approximately 120 isolates of native microflora were screened for their ability to inhibit the growth of *L. monocytogenes, Salmonella* Chester, *E. coli,* and *E. carotovora* subsp. *carotovora* on PAF. Six of them, designated, respectively, as strain AB1, AB2, AB3, AB4, A3, and D1, were capable of suppressing the growth of at least one pathogen (Fig. 1). All six strains were nonpectolytic and able to inhibit the growth of targeted pathogens or bacteria to different degrees as indicated by

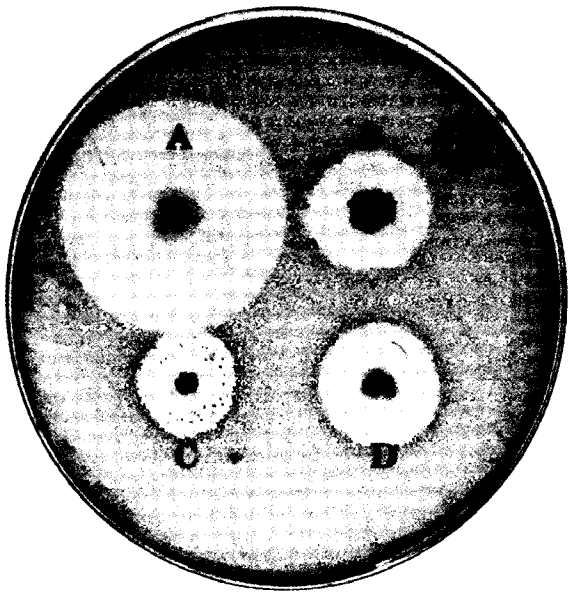

FIGURE 1. *Formation of growth inhibition zones against* L. monocytogenes *by four strains of antagonists. A, strain A3 (*P. fluorescens*); B, strain D1 (yeast); C, strain AB2 (*Bacillus mojavensis*); and D, strain AB3 (*Bacillus megaterium*).*

TABLE 5. *Comparison of growth inhibition zones produced by six strains of antagoinsts from fresh produce*[a]

Strain[b] designation	Scientific name	Growth inhibition zone (in mm) against:			
		L. monocytogenes	*E. coli*	*Salmonella* Chester	*Erwinia carotovora*
AB1	*Bacillus pumilus*	14	ND	ND	ND
AB2	*B. mojavensis*	4	3	ND	3
AB3	*B. megaterium*	6	4	ND	5
AB4	*P. aeruginosa*	17	12	ND	9
A3	*P. fluorescens*	11	8	ND	10
D1	Yeast	7	6	4	8

[a] Antagonists were grown on PAF at 28°C for 3 days. After that, each culture plate was exposed to chloroform vapor to kill the antagonists and then overlayed with water agar seeded with a targeted pathogen. The inhibition zone (in mm diameter) was measured from the edge of the antagonist colony. ND, not detected.

[b] Strains AB1 and AB4 were originated from clover seeds; strains AB2, AB3, and D1 from prepeeled baby carrots; and strain A3 from Romaine lettuce.

growth-inhibition zones on PAF ranging from 3 to 17 mm in diameter (Table 5). Strains AB1, AB2, and AB3 were identified as *Bacillus* species, strain AB4 as *Pseudomonas aeruginosa,* strain A3 as *Pseudomonas fluorescens,* and strain D1 was tentatively identified as a yeast mainly based on its cellular morphology. All six strains except strain AB1 inhibited the growth of *L. monocytogenes, E. coli,* and *E. carotovora* subsp. *carotovora* on PAF. However, only strain D1 suppressed the growth of *Salmonella* Chester. Strain AB1 inhibited the growth of *L. monocytogenes* but not other pathogens. We have previously shown that fluorescent pseudomonads are capable of suppressing the growth of *L. monocytogenes* by producing ion-chelating siderophores on PAF *(22).* Here we identified additional strains of native microflora including *Bacillus* species and yeast that are capable of inhibiting the growth of human and plant pathogens on agar medium.

Assays of strains A3 and D1 as potential biocontrol agents. Figure 2 shows that the growth of *L. monocytogenes* or *Salmonella* Chester on green pepper disks was not markedly affected by the presence of 100-fold higher concentration of native and uncharacterized flora. However, when pepper disks were coinoculated with a pathogen and strains A3 and D1, the growth of *L. monocytogenes* and *Salmonella* Chester on green pepper disks was reduced by approximately 2 logs and 1 log, respectively (Fig. 3). When pepper disks were coinoculated with *L. monocytogenes* and strain AB1, the growth of this pathogen was reduced by 2 logs. However, strain AB1 did not inhibit the growth of *Salmonella* Chester or *E. coli* on pepper disks. Strains AB2, AB3, and AB4 also reduced the growth of *L. monocytogenes* or *E. coli* on pepper disks by less than 1 log. A combination of strains A3 (*P. fluorescens*) and D1 (yeast) showed the most effect in reducing the growth of *L. monocytogenes, E. coli,* and *Salmonella* Chester on pepper disks and represented potential biocontrol agents for reducing the growth of these two pathogens on fresh produce products. Several strains of LAB have been shown to be effective in suppressing the growth of pathogens on ready-to-use vegetables *(28).* Recently, Janisiewicz et al. *(16)* also reported that a saprophytic *Pseudomonas syringae* strain (L-59-66) was effective in reducing the growth of decay pathogens and human pathogen *E. coli* O157:H7 on wounded apple

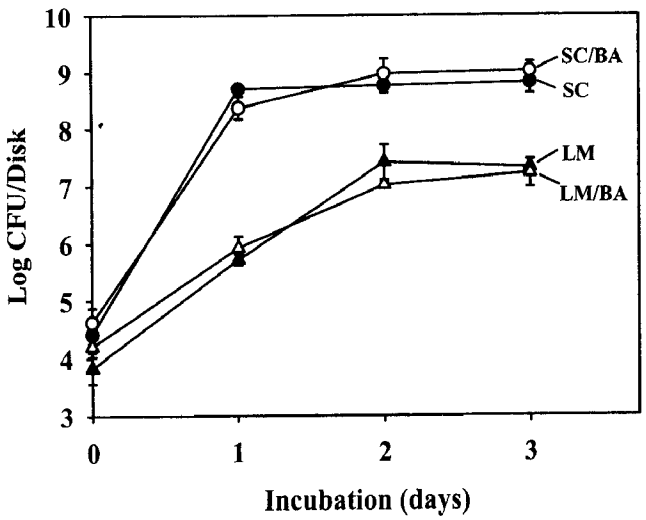

FIGURE 2. *Growth of* L. monocytogenes *(LM) and* Salmonella *Chester (SC) on green pepper disks in the presence or absence of excessive numbers of background bacteria (BA) prepared from green bell peppers.*

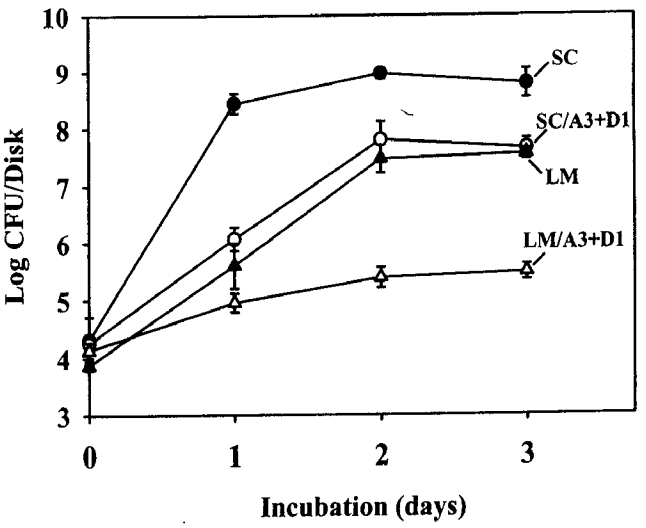

FIGURE 3. *Growth of* L. monocytogenes *(LM) and* Salmonella *Chester (SC) on green pepper disks in the presence or absence of two antagonistic strains, A3 and D1.*

fruits. We previously demonstrated that growth of *L. monocytogenes* on potato tuber slices was almost completely inhibited in the presence of certain strains of fluorescent soft-rotting pseudomonads *(22)*. The potential application of strains A3 and D1 as biopreservatives of fresh or fresh-cut salad vegetables is now being investigated. Such an application could extend the shelf life and safety of salad vegetables. However, the toxicological and environmental impacts associated with the application of these two biological agents and the economic benefit from the use of these new biological products need to be evaluated further.

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